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## Indian Standard

# SPECIFICATION FOR RESEARCH MICROSCOPE

UDC 535 822



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October 1969



#### AMENDMENT NO. 3 MAY 1983

TO

#### IS:5204-1969 SPECIFICATION FOR RESEARCH MICROSCOPE

## Alterations

(Page 10, clause 6.2.4):

a) heading - Substitute the following for the existing heading:

'Field of View Mumber (Field Mumber) of Eyepiece'.

b) Last sentence - Substitute the following matter for the existing:

'An alternative method for the measurement of field of view number shall be as given below:

A microscope fitted with an objective with magnification 40% or 45% and the eyepiece under test shall be focused on a stage micrometer. The exact magnification of the objective shall be determined according to the method given in Appendix J of IS:2754-1964\*. A stage micrometer normally has 1 mm divided into 100 divisions, thereby making each graduation equal to 0.01 mm. The maximum number of graduations on the stage micrometer which are seen with the eyepiece under test are noted. This number expressed in mm gives the diameter of the visual field of the microscope. The field of view number of the eyepiece is given by the formula:

Pield of view = field in mm X magnification of the objective.

(EDC 36)

### AMENDMENT NO. 2 SEPTEMBER 1980

#### TO

# IS: 5204-1969 SPECIFICATION FOR RESEARCH MICROSCOPE

#### Alterations

[ Page 6, clause 4.8.1(a) to (d)] — Substitute the following for the existing matter:

- 'a) 32 mm ( $5 \times NA 0.125$ ),
- b)  $16 \text{ mm} (10 \times \text{NA } 0.25)$ ,
- c) 4 mm (40  $\times$  NA 065) or (45  $\times$  NA 065), and
- d) 2 mm (90 × NA 125) or (100 × NA 1.25) oil immersion.

( Page 9, clause 6.1.3 ) — Substitute the following for the existing clause:

'6.1.3 In changing from lower magnification to next higher magnification or re-introducing the same objective by rotation of the nosepiece, the object at the centre of the field shall be well within the field of view.'

( Page 10, clause 6.2.6 ) — Substitute the following for the existing caluse:

'6.2.6 When observed under white light illumination, the image shall appear clear and well defined. The image shall be totally free from colour defects up to half the field of view and shall be reasonably free from colour up to two-thirds field of view.'

#### Addenda

( Page 6, clause 4.8.2 ) - Add the following new note after 4.8.2:

\*Note — The mechanical tube length of 160 mm is applicable only in case of monocular microscope while for "bī-nocular microscope the tube length may be up to 200 mm."

( Page 9, clause 6.1.1 ) — Add the following new note after 6.1.1:

'Note — The mechanical tube length of 160 mm is applicable only in case of monocular microscope while for bi-nocular microscope the tuble length may be up to 200 mm.'

(EDC 36)

### AMENDMENT NO. 1 DECEMBER 1979

TO

#### IS: 5204-1969 SPECIFICATION FOR RESEARCH MICROSCOPE

## <u>Alterations</u>

(Page 5, clause 4.6) - Substitute the following for the existing clause:

'4.6 Light Source - The microscope shall be equipped with a high intensity compact light source enclosed in a suitable housing complete with condensing lens, iris diaphragm and filters including ground glass for critical and Köhler illumination of the specimen.'

(Page 7, clause 4.9.1.1, line 2) - Substitute '0.01 mm' for '0.001 mm'.

(EDC 36)

# Indian Standard SPECIFICATION FOR RESEARCH MICROSCOPE

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# Indian Standard SPECIFICATION FOR RESEARCH MICROSCOPE

#### O. FOREWORD

- 0.1 This Indian Standard was adopted by the Indian Standards Institution on 8 July 1969, after the draft finalized by the Optical and Mathematical Instruments Sectional Committee had been approved by the Mechanical Engineering Division Council.
- **0.2** Research microscope is a more precise, convenient and a perfect form of general purpose microscope capable of being used for critical microscopic observations over prolonged periods. It is provided with arrangements for bright and dark field illuminations suitable for direct observation, photomicrography and projection of transparent specimens.
- 0.3 For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test, shall be rounded off in accordance with IS: 2-1960\*. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

#### 1. SCOPE

1.1 This standard covers the general and functional requirements of the research microscope.

#### 2. TERMINOLOGY

- 2.0 For the purpose of this standard, the definitions given in IS: 1399-1959† and the following shall apply.
- 2.1 Critical Illumination Illumination of the specimen, directly by light source focused on the plane of the object.
- 2.2 Kohler Illumination Illumination of the specimen, by the surface of an auxiliary lens used as a light source instead of the direct light source focusing on the plane of the object to illuminate the specimen with uniform, extended and an intense controlled illumination.

<sup>\*</sup>Rules for rounding off numerical values ( revised ).

<sup>†</sup>Glossary of terms used in optical technology.

- 2.3 Bright Field Illumination Illumination in which the specimen is illuminated with intense light which directly passes into the objective and the image is formed from direct, diffracted, reflected and refracted light by the specimen.
- 2.4 Dark Field Illumination Illumination in which no direct light is permitted to enter the objective and the image is formed entirely from diffracted, reflected and refracted light by the specimen.

#### 3. DIMENSIONS

3.1 The dimensions of the research microscope shall conform to IS: 3081-1965\*.

#### 4. GENERAL REQUIREMENTS

- **4.0** The research microscope shall conform to the requirements laid down in IS: 988-1959† and to the general requirements laid down in 3 of IS: 2754-1964‡.
- 4.1 Base The base shall be of heavy construction for stability and freedom from tremor.
- **4.2 Limb** The microscope shall be provided with a non-hinged or hinged type limb preferably in the form of an arc to allow large sized objects to be placed on the stage and also to facilitate carrying the microscope from place to place.
- 4.3 Stage The microscope shall be furnished with either a rectangular stage or a rotating centring object stage with device for crosswise displacement of the object.
- **4.3.1** Circular Stage The circular stage shall preferably be 125 mm in diameter to accommodate the standard slides of maximum dimension 50 mm × 76 mm as specified in IS: 3099-1965§.
- 4.3.2 Rectangular Stage The rectangular stage shall preferably be of dimension 120 mm × 125 mm to accommodate standard slides of maximum dimension 50 mm × 76 mm.

#### 4.4 Body Tube Attachments

- 4.4.1 The microscope shall be suitably designed to accommodate the following attachments:
  - a) Inclined binocular eyepiece tube for ease of observation and to provide stereoscopic relief,

<sup>\*</sup>Dimensions and marking of general purposes microscopes.

<sup>†</sup>General requirements for optical components.

tGeneral requirements for optical instruments.

Specification for slides and cover slips for microscopes.

- b) Straight monocular eyepiece tube for direct vision and for photomicrographic and projection purposes, and
- c) A combined straight and inclined tube to deviate a small percentage of image forming light into the inclined tube to facilitate observation of the image whilst the specimen is being photographed.
- 4.4.2 The straight tube shall be provided with a draw tube for the adjustment of the photomicrographic and projection attachments. The draw tube may be graduated.

#### 4.5 Illumination

- **4.5.1** The microscope shall be provided with facilities for critical as well as Kohler illumination.
- **4.5.2** Facilities shall also be provided for carrying out microscopical investigations under bright and dark fields with the help of suitable light sources and sub-stage condensers (see **4.6** and **4.7.3**).
- **4.5.3** A variable central opaque light stop shall be used to achieve dark field illumination. The stop shall be capable of completely preventing the light rays from passing directly into the objective.
- **4.6 Light Sources** The microscope shall be equipped with the following sources of illumination:
  - a) An extended uniformly illuminated surface, such as an opal bulb or frosted glass for low or even medium powers, enclosed in a suitable housing, complete with iris diaphragm and filter for visual microscopy of transparent specimen, and
  - b) A high intensity compact light source used in conjunction with lamp condensing lens for high powers and more exacting work.

#### 4.7 Condensing System

- **4.7.1** Lamp Condenser The sondenser lens shall be well-corrected so that it forms an image of uniform intensity of the source in the plane of an iris diaphragm mounted in the illuminating train.
- 4.7.2 Mirror A suitable substage plane and concave mirror shall be provided with the microscope. The concave mirror shall be able to concentrate a beam upon a small area of the object when the condenser is not in use.

#### **4.7.3** Substage Condensers

4.7.3.1 For bright field examination, the microscope shall be equipped with a suitable achromatic oil immersion condenser provided with a

detachable top lens which may be easily removed to increase the working distance as required in low power works.

- 4.7.3.2 For dark field examination, the microscope shall be furnished with a refracting or reflecting aspheric condenser capable of illuminating the object by a large aperture of rays not directly injected into the objective.
- 4.7.3.3 The numerical aperture of the condensers shall not be less than that of the highest power objective.
- 4.7.3.4 The condensers shall be complete with iris diaphragms and filter holders which may be swung out of the system when desired.
- 4.7.3.5 Rack and pinion arrangement shall be provided for up and down movement of the condenser. Suitable arrangement shall be made to restrict the upward movement up to a predetermined position so that the front lens of the condenser may not strike the stage-surface.

#### 4.8 Objectives

- **4.8.1** The microscope shall be provided with four achromatic, semi-apochromatic or apochromatic objectives. The equivalent focal length, preferred magnification and numerical aperture shall be as under:
  - a)  $16 \text{ mm} (10 \times \text{NA } 0.25)$ ,
  - b) 8 mm ( $20 \times NA \cdot 0.50$ ),
  - c) 4 mm ( $40 \times NA \ 0.65$ ) or ( $45 \times NA \ 0.65$ ), and
  - d) 2 mm ( $90 \times NA \cdot 1.25$ ) or ( $100 \times NA \cdot 1.25$ ) oil immersion.

Note - The values of numerical aperture specified are the minimum.

- **4.8.1.1** When required by the purchaser, the objective having focal length, preferred magnification and numerical aperture of 32 mm ( $5 \times NA \ 0.125$ ) may also be supplied.
- 4.8.1.2 A groove filled with red paint shall be provided on the oil immersion objective for quick identification.
- **4.8.2** The objectives shall be corrected for the mechanical tube length of 160 mm and cover slip thickness of 0.17 mm with tolerances of  $\pm$  1 mm and  $\pm$  0.015 mm respectively.
- 4.8.3 There shall be provision for the coarse and fine focusing movement of the objective relative to the stage and it shall be so restricted by a suitable slide protecting device that it may not, at any time damage the microslide placed on the stage.
  - 4.8.4 Suitable containers shall be provided for keeping objectives.

#### 4.9 Eyepieces

- 4.9.1 The microscope shall be provided with the eyepieces of following types and magnifications as required by the customer:
  - a) Huygenian:  $5 \times$ ,  $10 \times$  and  $15 \times$ ;
  - b) Ramsden:  $5 \times$  and  $15 \times$ ; and
  - c) Compensating:  $10 \times$ .
- **4.9.1.1** If required by the purchaser, an eyepiece with graticule and micrometer arrangement with measuring facility upto 0.001 mm may be provided.
- 4.9.2 For binocular vision, paired eyepieces of above types and magnifications shall be supplied.
- 4.10 Case The case carrying the microscope shall be made of well-seasoned wood. Teak ply or board may also be used. It shall be suitably padded from inside to eliminate the risk of shock during transportation. It shall be complete with lock and key arrangement, and a suitable locking screw for securing the microscope and a cross-piece to retain it in position during transit. The case shall be of a suitable design with a carrying handle at the top and suitable internal receptacles for holding the objectives, eyepieces and accessories. It shall contain a bag of activated silica gel to keep the interior moisture-free, and also a cleaning brush.

#### 5. FUNCTIONAL REQUIREMENTS

#### 5.1 Circular Stage

- 5.1.1 The hole in the stage shall be located centrally with respect to the microscope axis.
- 5.1.2 The circumference of the stage shall be graduated in degrees and fitted with a vernier having well-defined and distinct graduations. The vernier shall be so graduated that the amount of circular movement of the stage may be read up to at least six minutes of an arc.
- **5.1.3** The transverse and front to back movement of the stage shall be 70 mm and 30 mm respectively so that the entire area of maximum  $50 \text{ mm} \times 76 \text{ mm}$  slide may be examined.
- 5.1.4 Scales and verniers, with well-defined and distinct graduations, shall be provided to read the amount of crosswise movement. The verniers shall be so graduated that the amount of movement in either direction may be read to an accuracy of at least 0.1 mm.
- 5.1.5 The circular and crosswise movements of the stage shall be easy and smooth.

#### 5.2 Rectangular Stage

- 5.2.1 The hole in the stage shall be located centrally with respect to the microscope axis.
- **5.2.2** The transverse and front to back movement of the stage shall be 70 mm and 30 mm respectively so that the entire area of the maximum  $50 \text{ mm} \times 76 \text{ mm}$  slide may be examined.
- 5.2.3 Scales and verniers, with well-defined and distinct graduations, shall be provided to read the amount of crosswise movement. The verniers shall be so graduated that the amount of movement in either direction may be read to an accuracy of at least 0.1 mm. The tolerance between any two graduations shall be 0.01 mm.
- **5.2.4** The movement of the stage in two perpendicular directions shall be easy and smooth.

#### 5.3 Condensers

- 5.3.1 The iris diaphragms of lamp condenser and substage condenser shall open and close centrally and smoothly.
- **5.3.2** The circular and the up and down movement of the substage condenser shall be easy and smooth and shall remain unaffected by the load of accessories likely to be used.

#### 5.4 Objectives and Eyepieces

- **5.4.1** The coarse and fine focusing movement of the objective relative to the stage shall be smooth and even and shall remain unaffected by the load of accessories likely to be used.
- 5.4.2 The fine motion shall be 0.1 mm per revolution of the corresponding knob over a range of 1 to 2 mm.
- 5.4.3 The nosepiece shall be smooth in movement without any shake and shall not be displaced by any jerk.
- 5.4.4 The objectives and eyepieces shall be parfocal to within one quarter of a turn of the fine motion knob. It is essential that these parfocal distances be maintained at the values given in IS: 3081-1965\*.
- 5.4.5 The edge of the eyepiece stop shall appear in focus and shall be dull black.
- 5.5 Optical Axis The optical axis of the microscope shall coincide with the mechanical axis.

<sup>\*</sup>Dimensions and marking of general purposes microscopes.

5.6 Image — The image shall be well-defined with good contrast rendition and practically free from spurious colour effects, curvature of field over three-fourths of the field of view, and distortion.

#### 6. TESTS

#### 6.1 Mechanical

- **6.1.1** The mechanical tube length shall be within  $\pm 1$  mm of the standard nominal value of 160 mm.
- **6.1.2** The stage shall be checked for its squareness to the axis of the microscope maintaining the objective parfocal distance. The deviation from squareness shall not exceed five minutes of arc.
- **6.1.3** In changing from one objective to another or reintroducing the same objective by rotation of the nosepiece, the object at the centre of the field shall not appear displaced by more than 0.02 mm in the object plane in any direction.
- 6.1.4 Operation of the fine motion through its full range shall not appear to displace an object by more than 0.01 mm.
- **6.1.5** The centring of the condenser shall be checked by rotating it in its holder. The rotation of the image of a distant object formed by the condenser shall be within  $\pm 1$  mm.
- **6.1.6** Parfocality of the objectives and the eyepieces shall be within one quarter of a turn of the fine motion knob.

#### 6.2 Optical

**6.2.1** Magnification and Numerical Aperture of Objectives — The magnification and numerical aperture of objectives shall be measured by the methods specified in **4.2.2** and **4.2.3** respectively of IS: 2754-1964\*. The measured values shall not differ from the specified values by more than five percent.

#### 6.2.2 Resolving Power and Definition

6.2.2.1 Depending upon the numerical aperture of the objective, an appropriate test slide (diatom), illuminated by a beam of nearly the same numerical aperture, shall be observed with the microscope. The fine structure shall appear resolved and well-defined. (The emphasis on a well-defined image is for protection against misleading results due to spurious resolution.) The object shall be carefully and judiciously chosen

<sup>\*</sup>General requirements for optical instruments.

in the test diatom slide. The recommended diatoms are as follows:

 $\begin{array}{lll} \textit{Magnification/NA} & \textit{Recommended Diatoms} \\ \text{For } 10 \times /0.25 & \text{Navicula lyra} \\ \text{For } 20 \times /0.50 & \text{Navicula lyra} \\ \text{For } 40 \times /0.65 \text{ or } 45 \times /0.65 & \text{Pleurosigma angulatum} \\ \text{For } 90 \times /1.25 \text{ or } 100 \times /1.25 & \text{Amplipleura pellucida} \\ \end{array}$ 

- 6.2.2.2 Alternatively, the resolving power of the objective shall be measured by the method given in Appendix K of IS: 2754-1964\*. The measured value shall not be less than the theoretical value by more than five percent.
- **6.2.3** Eyepiece Magnification The equivalent focal length of eyepieces shall be measured by any of the methods given in Appendix C of IS: 988-1959†, and then the measurement of magnification shall conform to **4.2.5** of IS: 2754-1964\*.
- 6.2.4 Field Number of Eyepiece The eyepiece shall be removed from the microscope and the diaphragm shall be illuminated by diffused light from the eye-lens side. The diameter of the image of the diaphragm formed by the field lens shall be measured in millimetres with a measuring (travelling) microscope placed towards the field lens of the eyepiece. The value shall be in conformity with that calculated by the formula given in IS: 2754-1964\*.
- 6.2.5 Star Test for Definition The objective shall be tested separately for aberrations over three-fourths of the field of view by the examination of the intra-focal and extra-focal images of a 'Star' object; the latter being artificially produced by suitably illuminating the smallest mercury globbles on a piece of glass painted black, placed on the stage of the microscope under test. For observing the diffraction patterns thus formed on either side of the focus of the objective under test, an auxiliary testing microscope may be used in place of the eyepiece to provide a magnification higher than 15× in doubtful cases; otherwise normally a 15× eyepiece may be used.
- **6.2.6** Colour -- The image of a black or white test object shall appear free from colour when observed by illumination with white light over three-fourths of the field of view.
- 6.2.7 Curvature of Field The image shall be free from curvature of field to the extent of nearly three-fourths of the total field. A microslide having 2 mm divided into 200 parts or 1 mm divided into 100 parts shall be focused under the objective and nearly three-fourths of total number of

<sup>\*</sup>General requirements for optical instruments.

<sup>†</sup>General requirements for optical components.

graduations visible in the field of view shall appear well-defined and be symmetrically located in the field of view.

- 6.2.8 Distortion A counting chamber slide or a cross-line grating shall be focused in order to judge visually whether the image is free from distortion.
- **6.2.9** Clarity of the Field of View It shall be observed that no shadow hones along the periphery of the field of view are present (These may arise due to improper location of stray-light baffles).
- **6.2.10** Condition of Optics The optics shall be checked for conformity to the requirements as laid down in IS: 988-1959\*.

#### 7. MARKING

- 7.1 Each microscope shall be marked at a suitable place with the manufacturer's name or trade-mark and the serial number. If required by the purchaser, the microscope may also be marked with the year of manufacture.
- 7.2 The marking of the objectives and eyepieces shall be according to IS: 3081-1965†.
- 7.2.1 The microscope may also be marked with the ISI Certification Mark.

Note — The use of the ISI Certification Mark is governed by the provisions of the Indian Standards Institution (Certification Marks) Act, and the Rules and Regulations made thereunder. Presence of this mark on products covered by an Indian Standard conveys the assurance that they have been produced to comply with the requirements of that standard, under a well-defined system of inspection, testing and quality control during production. This system, which is devised and supervised by ISI and operated by the producer, has the further safeguard that the products as actually marketed are continuously checked by ISI for conformity to the standard. Details of conditions, under which a licence for the use of the ISI Certification Mark may be granted to manufacturers or processors, may be obtained from the Indian Standards Institution.

#### 8. PACKING AND PACKAGING

- **8.1** The microscope shall be free from all traces of foreign material and shall be delivered in a thoroughly clean and dry condition.
- 8.2 All the objectives shall be placed in cylindrical containers which, in turn, shall be held in a wooden receptacle fixed or sliding inside the wooden case of the microscope. The eyepieces shall be put in their respective positions in the same receptacle. Other accessories shall be placed in their respective places. During transit, the objectives, eyepieces and other accessories shall be well secured in position.

<sup>\*</sup>General requirements for optical components.

<sup>†</sup>Dimensions and marking of general purposes microscopes.

- 8.3 The straight tube fitted in the microscope shall be brought to its lowest position by means of the coarse focusing knob before being put into the case. The binocular eyepiece tube and the combined straight and inclined tube shall be suitably secured in their respective positions.
- 8.4 The filters, if provided, and the mirror shall be wrapped in a clean, dry and acid-free tissue paper and shall be suitably retained in position.
- 8.5 The condenser unit shall also be wrapped in a clean, dry and acid-free tissue paper and suitably secured in its position.
- **8.6** The bulbs shall be placed in a suitable container which shall be well secured in the case.
- 8.7 The microscope shall then be placed in the case and held in position securely by suitable arrangement.
- **8.8** A bag containing 300 g of activated silica gel shall be suitably placed inside the case.
- 8.9 The empty spaces, if any, shall be filled with suitable size bags or pillows or both stuffed with suitable packing material.
- 8.10 The case along with its contents, shall be locked during transit and the keys attached to the handle.
- **8.11** The case shall then be packed in a packing box lined with waterproof paper, using suitable cushioning material. The lid shall be screwed down.
- 8.12 The package shall be marked with the description, the number of contents, the standard symbol for indicating fragile contents and the symbol for 'THIS WAY UP' according to IS: 1286-1967\*, together with the legend 'INSTRUMENT, HANDLE WITH CARE' in red.

<sup>\*</sup>Pictorial markings for handling of goods in general (first revision).

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